Intralesional Bleomycin Treatment for an Oral Squamous Cell Carcinoma in Indo-Pacific Bottlenose Dolphin (*Tursiops aduncus*)

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ABSTRACT

This study describes the case of an Indo-Pacific bottlenose dolphin (*Tursiops aduncus*) that experienced clinical manifestations since March 2018. Biopsy samples from his oral mucous membrane were collected in November 2018, confirming the diagnosis of oral squamous cell carcinoma (OSCC). The dolphin was administered the nonsteroidal anti-inflammatory drug meloxicam, however, the lesion continued to gradually deteriorate. Therefore, intralesional (ILES) bleomycin was administered. After several months, the lesion gradually improved without serious side effects. This case is the first report of ILES bleomycin treatment for OSCC in the Indo-Pacific bottlenose dolphin.

Key words: Indo-Pacific bottlenose dolphin, intralesional bleomycin, oral squamous cell carcinoma

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Oral squamous cell carcinoma (OSCC) is a malignant tumor commonly reported in cats and dogs. However, there are a few reports of the disease in Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) [1] and bottlenose dolphins (*Tursiops truncatus*) as oral presentation in international association for aquatic animal medicine (IAAAM) [2, 3]. Surgery, chemotherapy, radiotherapy, and nonsteroidal anti-inflammatory drugs are the treatment modalities.

In this case, the aquarium-maintained Indo-Pacific bottlenose dolphin is a male, over 46 years old captive at the end of November 2020, and its body weight was 186.0 kg in September 2020.

Since March 2018, the dolphin had developed a small, red-like inflamed lesion, which was suspected to result from a fishbone prick (Fig.1). Therefore, the dolphin received topical treatments with ointments, including triamcinolone acetonide (Bristol-Myers Squibb Company Tokyo, Japan),

hinokitiol, hydrocortisone acetate and ethyl aminobenzoate (Showa Yakuhin Kako Co., Ltd., Tokyo, Japan), beclomethasone dipropionate (Teijin Pharma Limited, Tokyo, Japan), and povidone-iodine (Shionogi & Co., Ltd., Osaka, Japan). The dolphin was also administered antibiotics such as azithromycin hydrate (Pfizer Japan Inc., Tokyo, Japan) and tranexamic acid (Daiichi Sankyo Co., Ltd., Tokyo, Japan), which exert anti inflammatory effects. Although the clinical treatment was continued for several months, the lesion did not improve.

Four samples of the lesion with of approximately 5 × 5 mm in size were collected in November 2018 using sharp spoon forceps for otolaryngology or scalpel and fixed in 10% phosphate-buffered formalin solution. The histopathology results (North Lab, Hokkaido, Japan) identified the lesion as being OSCC (Fig. 2a, b).

From December 2018, the dolphin received systemic oral administration of meloxicam [1, 4] once a week or once every five days for one month. The dolphin received seven courses with drip infusion, monoammonium glycyrrhizinate, glycine, and L-cysteine hydrochloride hydrate (Nipro, Osaka, Japan) to improve the liver function and famotidine (Astellas Pharma,

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Tokyo, Japan) to prevent side effects. The dolphin initially responded to the treatment; however, the lesion demonstrated repeated aggression and remission. Lastly, the tumor was estimated to be deeper, with approximately $5.0 \times 2.5 \times 2.0$ cm in size (Fig.3). Therefore, ILES bleomycin was administrated next given the expected side effects are in general mild, consisting of wound formation with bone exposure that could be solved with antibiotics, mild tissue reactions, local swelling and local infection [5, 6].

Before the treatment, the dolphin was evaluated for complete blood count (poch-100iV Diff, Sysmex Corporation, Hyogo, Japan), blood serum biochemical analysis (DRI-CHEM 7000V,

Fig.1 The dolphin had developed a small red lesion, which was suspected to result from a fishbone prick on March 17th 2018.

Fig. 2 The site of biopsy

a : We took four samples using sharp spoon forceps for otolaryngology or scalpel on November 18th 2018.

b : The result of pathology examination from area 2 and 4 (Fig2a). The tissue examined shows proliferation of atypical epithelial cells with irregular islands, cords, and trabeculae and invade to the adjacent tissue. The neoplastic cells have abundant eosinophilic cytoplasm and round nuclei with prominent nucleoli. And some of these cells show mild keratinization. The mitotic figures are few.

Fujifilm Corporation, Tokyo, Japan), computer tomography scan (SOMATOM Definition AS+, Siemens Healthineers, Tokyo, Japan), ultrasound (ARIETTA Prologue, Hitachi Ltd., Tokyo, Japan), and thermography (Thermo Shot, Nippon Avionics Co., Ltd., Kanagawa, Japan). All the tests showed that the dolphin was healthy, without metastasis, and with normal appetitend behavior.

Before the ILES procedure, ethyl aminobenzoate was applied (Bee Brand Medico Dental Co., Ltd., Osaka, Japan) and lidocaine hydrochloride was injected (Aspen Japan, Co., Ltd., Tokyo, Japan) inside and around the lesion under topical anesthesia. Four syringes were prepared to draw the bleomycin (Nippon

Fig. 3 The first day of the procedure on June 20th 2020.

Table 1 Hematology and serum chemistry of this dolphin during intralesional bleomycin treatment³⁾

	Range of this dolphin ¹⁾	20/06/23	20/06/30	20/07/07	20/07/14	20/07/21	20/08/04 ²⁾	20/08/11 ²⁾	20/08/23	20/09/10	20/09/21	20/10/05	20/10/20
WBC ⁴⁾ (/ul)	2,100-5,400	3,800	4,200	4,100	3,600	4,500	7,700	6,100	3,500	4,800	2,700	2,700	3,675
RBC ($\times 10^4/\mu\text{l}$)	292-341	307.0	324.0	320.0	332.0	315.0	330.0	322.0	311.0	326.0	336.0	331.0	327.0
HGB (g/dl)	13.4-16.6	15.0	15.8	15.4	16.1	15.2	15.6	15.4	14.5	15.5	16.2	15.6	15.8
HCT (%)	43-49	44.0	47.0	44.0	46.0	45.0	46.0	46.0	44.0	44.0	48.0	44.5	45.0
Na (mmol/l)	149-159	156	151	154	156	158	155	155	155	158	156	152	152
K (mmol/l)	3.2-4.5	3.8	4.3	3.6	3.8	3.7	3.7	3.5	3.5	3.6	3.3	3.8	3.6
Cl (mmol/l)	115-127	119	114	118	120	123	118	120	120	122	119	115	116
LDH (U/l)	484-762	560	831	557	529	562	470	482	547	541	547	597	616
CPK (U/l)	69-162	98	83	95	95	103	67	79	89	110	90	103	116
GLU (mg/dl)	67-122	98	94	104	91	102	106	100	89	103	112	93	93
ALB (g/dl)	3.7-4.6	4.2	4.8	4.4	4.4	4.2	4.3	4.1	4.2	4.2	4.5	4.3	4.3
Tcho (mg/dl)	117-202	158	156	156	153	137	141	125	133	129	152	149	144
TG (mg/dl)	11-121	25	26	27	15	32	19	29	15	20	36	30	16
TP (g/dl)	11-121	8.4	9.1	8.5	8.9	8.3	8.6	8.8	8.6	8.6	8.8	8.8	8.9
ALT (U/l)	34-91	44	53	43	53	53	50	51	61	55	65	70	85
AST (U/l)	164-252	175	198	178	194	195	185	187	207	211	221	246	264
ALP (U/l)	295-767	651	617	569	627	583	519	502	623	511	581	581	778
TBil (mg/dl)	0.1-0.3	0.1	1.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.1
Ca (mg/dl)	7.5-9.2	8.6	8.4	9.0	8.8	8.7	8.1	8.6	8.3	8.5	8.8	8.8	8.4
IP (mg/dl)	4.4-6.3	5.2	4.7	5.7	6.6	5.8	5.5	5.0	5.5	5.3	5.1	5.7	5.0
BUN (mg/dl)	49.9-76.7	54.5	53.2	53.6	51.1	56.6	46.6	50.1	52.8	55.7	57.7	52.7	55.1
CRE (mg/dl)	1.3-2.1	1.83	1.70	1.77	1.84	1.69	1.60	1.60	1.68	1.65	1.61	1.63	1.44
UA (mg/dl)	0.1-0.8	0.3	0.2	0.2	0.3	0.3	0.2	0.2	0.2	0.2	0.2	0.3	0.4
NH ₃ ($\mu\text{g/dl}$)	31-59	35	50	50	44	32	62	42	57	29	42	31	39
GLOB (g/dl)	4-4.9	4.2	4.3	4.1	4.5	4.1	4.3	4.7	4.4	4.4	4.3	4.5	4.6
Mg (mg/dl)	1.7-2.1	1.8	1.7	1.8	1.8	1.8	1.7	1.7	1.8	1.6	1.7	1.7	1.8
Fib (mg/dL)	201-445	268	217	243	250	218	323	410	246	230	252	280	280

¹⁾ Range of this dolphin: 45 samples from 2016-2020, when the dolphin was clinically healthy.

²⁾ 20/08/04-20/08/11, the dolphin was received antibiotics because of bitten.

³⁾ Brief extract version taken from blood samples during this treatment.

⁴⁾ WBC, white blood cell count; RBC, red blood cell; HGB, hemoglobin content; HCT, hematocrit; Na, sodium; K, potassium; Cl, chloride; LDH, lactate dehydrogenase; CPK, creatine kinase; GLU, glucose; ALB, albumin; Tcho, total cholesterol; TG, total triglyceride; TP, total protein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; TBil, total bilirubin; Ca, calcium; IP, inorganic phosphorus; BUN, blood urea nitrogen; CRE, creatinine; UA, uric acid; NH₃, ammonia; GLOB, globulin; Mg, magnesium; Fib, fibrinogen

Kayaku Co., Ltd., Tokyo, Japan) into a 5 ml lock syringe with a 25 G needle and extension tube (200 mm length). Each syringe contained 2.5 ml of bleomycin (0.96 U/ml) to avoid the use of the same needle. The maximum dose of bleomycin was of 9.6 U with a reference dosage of 9.6 U/m², administered to dogs [7, 8]. Bleomycin was injected inside and around the tumor without leakage [5], thus the dose of bleomycin that we used was 5.2 U–9.6 U in one procedure. After removing the needle, dry gauze was used to apply slight pressure onto the injection area to allow hemostasis. In case of hemorrhage, cotton swab moistened with 0.1% adrenaline solution (Daiichi Sankyo) was used as compression hemostasis treatment. The duration of the procedure was approximately 10 min. Afterwards the behavior of the dolphin and occurrence of hemorrhage were monitored for one hour. The protocol was performed once a week for five

weeks, and the dolphin received two courses in June 2020 and September 2020. Blood samples were collected once or twice a week to monitor the health status of the dolphin (Table 1). Based on normal range values calculated from 45 blood samples when no abnormality was found from 2016 to 2020. Hematology data of the bottlenose dolphin were also used as reference [9]. Overall, blood test results at all assessment time points, as well as the appetite and behavior were normal.

After several months on this regimen, the lesions became smaller from the edge, the granulates smoothed gradually, fewer hemorrhagic events occurred, and the mucosa expanded, especially after the second treatment administration. (Fig 4a, b). The injection site showed slight reaction and redness for a few days.

This was the first case of ILES bleomycin treatment for OSCC

Fig. 4 November 15th 2020, improved the lesion and expanding of the dolphin's mucosa.

in the Indo-Pacific bottlenose dolphin. These data suggest that this procedure can be used to bypass the risk of general anesthesia and effectively treat OSCC without serious side effects. Nevertheless, the lesion persisted; therefore, new attempts of the ILES bleomycin treatment will be explored.

CONFLICT OF INTEREST

The authors report no conflict of interest.

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症例報告 腫瘍学

口腔扁平上皮癌に罹患したミナミバンドウイルカ (*Tursiops aduncus*) のブレオマイシン腫瘍内投与

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要 約

2018 年 3 月より当館で飼育しているミナミバンドウイルカの口腔内粘膜に臨床症状が認められ, 同年 11 月に生検検査を行ったところ口腔内扁平上皮癌と診断された。非ステロイド性抗炎症剤メロキシカムを投与したが, 最終的に悪化した為, ブレオマイシンの腫瘍内投与を実施した。数か月後, 深刻な副作用なく患部は徐々に良化した。これは, ミナミバンドウイルカに対するブレオマイシン腫瘍内投与の初症例である。

キーワード: 口腔扁平上皮癌, ブレオマイシン腫瘍内投与, ミナミバンドウイルカ

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